1: Nature. 1997 Feb 20;385(6618):733-6. Related Articles, Links

Erratum in:
- Nature 1997 Apr 17;386(6626):738.

Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-alpha.

Moss ML, Jin SL, Milla ME, Bickett DM, Burkhart W, Carter HL, Chen WJ, Clay WC, Didsbury JR, Hassler D, Hoffman CR, Kost TA, Lambert MH, Leesnitzer MA, McCauley P, McGeehan G, Mitchell J, Moyer M, Pahel G, Rocque W, Overton LK, Schoenen F, Seaton T, Su JL, Becherer JD, et al.

Department of Molecular Biochemistry, Glaxo Wellcome Research and Development Inc., Research Triangle Park, North Carolina 27709, USA.

Tumour-necrosis factor-alpha (TNF-alpha) is a cytokine that contributes to a variety of inflammatory disease states. The protein exists as a membrane-bound precursor of relative molecular mass 26K which can be processed by a TNF-alpha-converting enzyme (TACE), to generate secreted 17K mature TNF-alpha. We have purified TACE and cloned its complementary DNA. TACE is a membrane-bound disintegrin metalloproteinase. Structural comparisons with other disintegrin-containing enzymes indicate that TACE is unique, with noteable sequence identity to MADM, an enzyme implicated in myelin degradation, and to KUZ, a Drosophila homologue of MADM important for neuronal development. The expression of recombinant TACE (rTACE) results in the production of functional enzyme that correctly processes precursor TNF-alpha to the mature form. The rTACE provides a readily available source of enzyme to help in the search for new anti-inflammatory agents that target the final processing stage of TNF-alpha production.

PMID: 9034191 [PubMed - indexed for MEDLINE]

1: Science. 1996 Aug 30;273(5279):1227-31. Related Articles, Links

KUZ, a conserved metalloprotease-disintegrin protein with two roles in Drosophila neurogenesis.

Rooke J, Pan D, Xu T, Rubin GM.

Department of Genetics, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, CT 06536, USA.

During neurogenesis in Drosophila both neurons and nonneuronal cells are produced from a population of initially equivalent cells. The kuzbanian (kuz) gene described here is essential for the partitioning of neural and nonneuronal cells during development of both the central and peripheral nervous systems in Drosophila. Mosaic analyses indicated that kuz is required for cells to receive signals inhibiting the neural fate. These analyses further revealed that the development of a neuron requires a kuz-mediated positive signal from neighboring cells. The kuz gene encodes a metalloprotease-disintegrin protein with a highly conserved bovine homolog, raising the possibility that kuz homologs may act in similar processes during mammalian neurogenesis.

PMID: 8703057 [PubMed - indexed for MEDLINE]

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1: J Biol Chem. 1993 Jul 25;268(21):15343-6. Related Articles, Links

Structure of a non-peptide inhibitor complexed with HIV-1 protease. Developing a cycle of structure-based drug design.

Rutenber E, Fauman EB, Keenan RJ, Fong S, Furth PS, Ortiz de Montellano PR, Meng E, Kuntz ID, DeCamp DL, Salto R, et al.

Department of Biochemistry and Biophysics, University of California, San Francisco 94143-0448.

A stable, non-peptide inhibitor of the protease from type 1 human immunodeficiency virus has been developed, and the stereochemistry of binding defined through crystallographic three-dimensional structure determination. The initial compound, haloperidol, was discovered through computational screening of the Cambridge Structural Database using a shape complementarity algorithm. The subsequent modification is a non-peptidic lateral lead, which belongs to a family of compounds with well characterized pharmacological properties. This thioketal derivative of haloperidol and a halide counterion are bound within the enzyme active site in a mode distinct from the observed for peptide-based inhibitors. A variant of the protease cocrystallized with this inhibitor shows binding in the manner predicted during the initial computer-based search. The structures provide the context for subsequent synthetic modifications of the inhibitor.

PMID: 8340363 [PubMed - indexed for MEDLINE]

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1: Biochemistry. 1991 Jul 30;30(30):7369-72.

Three-dimensional structure of echistatin, the smallest active RGD protein.

Saudek V, Atkinson RA, Pelton JT.

Marion Merrell Dow Research Institute, Strasbourg, France.

Echistatin is a 49 amino acid protein isolated from the venom of a viper (Echis carinatus) and is one of the smallest natural adhesive ligands that interacts with integrin-type receptors through an Arg-Gly-Asp (RGD) sequence. The structure of echistatin in aqueous solution has been determined by nuclear magnetic resonance spectroscopy. Nuclear Overhauser spectra yielded 490 interatomic distance constraints, which were used in distance geometry calculations. The chain is shown to fold in a series of irregular loops to form a rigid core stabilized by four cystine cross-links. From this core an irregular hairpin and the C-terminus protrude. The core and the hairpin are further stabilized by a network of hydrogen bonds. The RGD sequence is located in a mobile loop at the tip of the hairpin. The mobility and its significance for activity are discussed.

PMID: 1854743 [PubMed - indexed for MEDLINE]

1: Biochemistry. 1996 Jun 25;35(25):8216-25.

ACS PUBLICATIONS

Human pro-tumor necrosis factor is a homotrimer.

Tang P, Hung M-C, Klostergaard J.

Department of Tumor Biology, University of Texas, M. D. Anderson Cancer Center, Houston, 77030, USA.

The structure of human transmembrane pro-TNF-alpha was studied both in intact cell systems and in an in vitro translation system. In intact cell systems (LPS-induced THP-1 and TNF cDNA-transfected COS-7), a trimer of pro-TNF was detected after chemical cross-linking based on its molecular weight in Western blotting analysis. The trimer was shown to be a TNF-specific protein and could be partially cleaved to 26-kDa pro-TNF monomers by cleaving the cross-linkers. The trimeric structure was assembled intracellularly, because it could be detected in both the in vitro microsomal translation system and in THP-1 cells coincident with the appearance of pro-TNF in the cell lysate, prior to secretion of mature TNF. To further analyze the relationship between the trimeric structure and the biological activity of pro-TNF, we characterized several noncleavable pro-TNF deletion mutants. We observed a correlation between expression of TNF cytotoxicity in a juxtacrine fashion and detection of trimer. Thus, human pro-TNF-alpha, like the secreted mature TNF-alpha, has trimeric structure which is assembled intracellularly before transport to the cell surface and is apparently required for mediating its biologic activity.

PMID: 8679576 [PubMed - indexed for MEDLINE]

1: Biochemistry. 1996 Jun 25;35(25):8226-33. Related Articles, Links

ACS PUBLICATIONS

Length of the linking domain of human pro-tumor necrosis factor determines the cleavage processing.

Tang P, Hung M-C, Klostergaard J.

Department of Tumor Biology, University of Texas M. D. Anderson Cancer Center, Houston, 77030, USA.

Several studies have indicated that only one cleavage site (Ala-1/Val+1) is involved in the release of mature TNF from human pro-TNF, whereas others have suggested that the linking sequence (residues -20 to -1) may be important. We previously demonstrated that a pro-TNF deletion mutant, delta -20- -1, was able to form a trimeric structure and mediate TNF cytotoxicity in a juxtacrine fashion without releasing mature TNF. We constructed seven mutants with smaller deletions within this region. Three 15-residue deletion mutants, delta -20- -6, delta -15- -1 and delta -20- -16, -10- -1, were noncleavable, although able to form a trimer and to mediate cytotoxicity through cell-to-cell contact. Three five- or ten-residue deletion mutants, delta -20- -16, delta -10- -1, and delta -5-, -1, behaved like the wild-type TNF; all formed a trimer and released mature TNF. These results suggested that in pro-TNF (1) the number of residues between the base of the trimer and the plasma membrane determines accessibility of the cleavage site to the pro-TNF processing enzyme(s) since small deletions did not block cleavage whereas large ones did regardless of the presence of the native cleavage site (-1/+1), (2) the native cleavage site is not sufficient for releasing mature TNF because mutant delta -20- -6, in which the native cleavage site was intact, was noncleavable, and (3) alternative cleavage site(s) may exist since mutants delta -10- -1 and delta -5- -1, which lack the native cleavage site, were cleavable.

PMID: 8679577 [PubMed - indexed for MEDLINE]

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1: Dev Biol. 1996 Dec 15;180(2):389-401. Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

ADAMs in fertilization and development.

Wolfsberg TG, White JM.

National Center for Biotechnology Information, National Institutes of Health, Bethesda, Maryland, 20894, USA.

Publication Types:
- Review
- Review, Academic

PMID: 8954712 [PubMed - indexed for MEDLINE]

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1: Proc Natl Acad Sci U S A. 1994 Aug 30;91(18):8447-51. Related Articles, Links

Structural interaction of natural and synthetic inhibitors with the venom metalloproteinase, atrolysin C (form d).

Zhang D, Botos I, Gomis-Ruth FX, Doll R, Blood C, Njoroge FG, Fox JW, Bode W, Meyer EF.

Department of Biochemistry and Biophysics, Texas A&M University, College Station 77843.

The structure of the metalloproteinase and hemorrhagic toxin atrolysin C form d (EC 3.4.24.42), from the venom of the western diamondback rattlesnake Crotalus atrox, has been determined to atomic resolution by x-ray crystallographic methods. This study illuminates the nature of inhibitor binding with natural (< Glu-Asn-Trp, where < Glu is pyroglutamic acid) and synthetic (SCH 47890) ligands. The primary specificity pocket is exceptionally deep; the nature of inhibitor and productive substrate binding is discussed. Insights gained from the study of these complexes facilitate the design of potential drugs to treat diseases where matrix metalloproteinases have been implicated, e.g., arthritis and tumor metastasis.

PMID: 8078901 [PubMed - indexed for MEDLINE]

1: Crit Rev Immunol. 1996;16(1):1-11. Related Articles, Links

Tumor necrosis factor: function, release and clearance.

Bemelmans MH, van Tits LJ, Buurman WA.

Department of Surgery, University Hospital Maastricht, The Netherlands.

Tumor Necrosis Factor (TNF) is a multifunctional cytokine. It plays an important role in the pathophysiology of several diseases. Recently, it has been discovered that TNF is circulating in two different forms, a bioactive form and an immunologically detectable form. These two forms of TNF show different clearance kinetics. The immunological form is supposed to be an inactivated TNF protein. For this inactivation, proteolytic degradation or TNF binding by inactivating proteins is necessary. In this review we have focused on TNF inactivation by TNF binding proteins. Recent data show that there are soluble TNF receptors circulating which can bind and inactivate TNF. These receptors are membrane-bound TNF receptors which have been proteolytically cleaved from the cell membrane. Two TNF receptors are circulating, the soluble TNF receptor of 55 kDa (P55) and the receptor of 75 kDa (P75). The receptors are held responsible not only for inactivation of the TNF, but also for the clearance of TNF. Recent data show that the kidney is the most important organ for TNF clearance, followed by the liver. All other organs are of less importance. In this review, function, release, and clearance of TNF are discussed.

Publication Types:
- Review
- Review, Academic

PMID: 8809470 [PubMed - indexed for MEDLINE]

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1: Biochem Biophys Res Commun. 1996 Aug 14;225(2):400-5. Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Relaxed specificity of matrix metalloproteinases (MMPS) and TIMP insensitivity of tumor necrosis factor-alpha (TNF-alpha) production suggest the major TNF-alpha converting enzyme is not an MMP.

Black RA, Durie FH, Otten-Evans C, Miller R, Slack JL, Lynch DH, Castner B, Mohler KM, Gerhart M, Johnson RS, Itoh Y, Okada Y, Nagase H.

Immunex Corporation, Seattle, Washington 98101, USA.

Tumor necrosis factor-alpha is released from cells by a proteolytic cleavage. Previous work suggested that a specific, non-matrix metalloproteinase carries out this cleavage, but matrix metalloproteinases have also been implicated. In this paper, we report that none of the matrix metalloproteinases tested cleaved peptide substrates as specifically as the non-matrix metalloproteinase. A matrix metalloproteinase did process tumor necrosis factor-alpha extracted from COS cells, but neither tissue inhibitor of metalloproteinases-1 nor -2 blocked tumor necrosis factor-alpha processing by human monocytes. Moreover, tissue inhibitor of metalloproteinases-1 had at most a partial effect on the in vivo release of the cytokine in mice. We conclude that a non-matrix metalloproteinase is the major physiological tumor necrosis factor-alpha convertase.

PMID: 8753775 [PubMed - indexed for MEDLINE]

1: Nature. 1997 Feb 20;385(6618):729-33. Related Articles, Links

A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP.

Immunex Corporation, Seattle, Washington 98101, USA. rblack@immunex.com

Mammalian cells proteolytically release (shed) the extracellular domains of many cell-surface proteins. Modification of the cell surface in this way can alter the cell's responsiveness to its environment and release potent soluble regulatory factors. The release of soluble tumour-necrosis factor-alpha (TNF-alpha) from its membrane-bound precursor is one of the most intensively studied shedding events because this inflammatory cytokine is so physiologically important. The inhibition of TNF-alpha release (and many other shedding phenomena) by hydroxamic acid-based inhibitors indicates that one or more metalloproteinases is involved. We have now purified and cloned a metalloproteinase that specifically cleaves precursor TNF-alpha. Inactivation of the gene in mouse cells caused a marked decrease in soluble TNF-alpha production. This enzyme (called the TNF-alpha-converting enzyme, or TACE) is a new member of the family of mammalian adamalysins (or ADAMs), for which no physiological catalytic function has previously been identified. Our results should facilitate the development of therapeutically useful inhibitors of TNF-alpha release, and they indicate that an important function of adamalysins may be to shed cell-surface proteins.

PMID: 9034190 [PubMed - indexed for MEDLINE]

1: Inflamm Res. 1997 Jun;46(6):211-5. Related Articles, Links

[SpringerLink] SUBSCRIBER ARTICLE

Anti-arthritic activity of hydroxamic acid-based pseudopeptide inhibitors of matrix metalloproteinases and TNF alpha processing.

DiMartino M, Wolff C, High W, Stroup G, Hoffman S, Laydon J, Lee JC, Bertolini D, Galloway WA, Crimmin MJ, Davis M, Davies S.

SmithKline Beecham Pharmaceuticals (UW2109), King of Prussia, PA 19406, USA.

OBJECTIVE AND DESIGN: The effects of two hydroxamate inhibitors of metalloproteinase and tumor necrosis factor alpha (TNF alpha) processing on endotoxin-induced plasma TNF alpha and arthritic lesions in adjuvant-induced arthritic (AA) rats were determined. MATERIAL AND TREATMENT: BB-1101 and BB-1433 were administered orally twice daily to AA Lewis rats with an established disease (days 13 to 22). AA rats (day 16) or normal rats were injected with bacterial endotoxin and plasma levels of TNF alpha were also determined. METHODS: Hindpaw swelling was measured plethysmographically. Bone degradation was determined by radiography and bone mineral densitometry. TNF alpha was quantified using a sandwich ELISA. RESULTS: The hydroxamic-acid pseudopeptides inhibited plasma. TNF alpha levels in vivo and significantly reduced swelling and bone degradation of the tibiotarsal joints of AA rats in the range of 10-50 mg/kg given orally ($p < 0.01$ by Student's t-test). CONCLUSIONS: Thus, these novel compounds offer a new disease modifying therapy for arthritis and the results also suggest that inhibition of TNF alpha production may contribute, at least in part, to their anti-arthritic activity.

PMID: 9243304 [PubMed - indexed for MEDLINE]

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1: J Mol Graph. 1993 Jun;11(2):134-8, 127-8. Related Articles, Links

SETOR: hardware-lighted three-dimensional solid model representations of macromolecules.

Evans SV.

Department of Biochemistry, University of British Columbia, Vancouver, Canada.

SETOR is designed to exploit the hardware lighting capabilities of the IRIS-4D series graphics workstations to render high-quality raster images of macromolecules that can undergo rotation and translation interactively. SETOR can render standard all-atom and backbone models of proteins or nucleic acids, but focuses on displaying protein molecules by highlighting elements of secondary structure. The program has a very friendly user interface that minimizes the number of input files by allowing the user to interactively edit parameters, such as colors, lighting coefficients, and descriptions of secondary structure via mouse activated dialogue boxes. The choice of polymer chain representation can be varied from standard vector models and van der Waal models, to a B-spline fit of polymer backbones that yields a smooth ribbon that approximates the polymer chain, to strict Cardinal splines that interpolate the smoothest curve possible that will precisely follow the polymer chain. The program provides a photograph mode, save/restore facilities, and efficient generation of symmetry-related molecules and packing diagrams. Additionally, SETOR is designed to accept commands and model coordinates from the standard input stream, and to control standard output. Ancillary programs provide a method to interactively edit hardcopy plots of all vector and many solid models generated by SETOR, and to produce standard HPGL or PostScript files. Examples of figures rendered by SETOR of a number of macromolecules of various classes are presented.

PMID: 8347566 [PubMed - indexed for MEDLINE]

1: Cell. 1981 Jan;23(1):175-82. Related Articles, Links

SV40-transformed simian cells support the replication of early SV40 mutants.

Gluzman Y.

CV-1, an established line of simian cells permissive for lytic growth of SV40, were transformed by an origin-defective mutant of SV40 which codes for wild-type T antigen. Three transformed lines (COS-1, -3, -7) were established and found to contain T antigen; retain complete permissiveness for lytic growth of SV40; support the replication of tsA209 virus at 40 degrees C; and support the replication of pure populations of SV40 mutants with deletions in the early region. One of the lines (COS-1) contains a single integrated copy of the complete early region of SV40 DNA. These cells are possible hosts for the propagation of pure populations of recombinant SV40 viruses.

PMID: 6260373 [PubMed - indexed for MEDLINE]

1: EMBO J. 1993 Nov;12(11):4151-7. Related Articles, Links

First structure of a snake venom metalloproteinase: a prototype for matrix metalloproteinases/collagenases.

Gomis-Ruth FX, Kress LF, Bode W.

Max-Planck-Institut fur Biochemie, Martinsried, Germany.

Adamalysin II, a 24 kDa zinc endopeptidase from the snake venom of Crotalus adamanteus, is a member of a large family of metalloproteinases isolated as small proteinases or proteolytic domains of mosaic haemorrhagic proteins from various snake venoms. Homologous domains have recently been detected in multimodular mammalian reproductive tract proteins. The 2.0 A crystal structure of adamalysin II reveals an ellipsoidal molecule with a shallow active-site cleft separating a relatively irregularly folded subdomain from the calcium-binding main molecular body composed of a five-stranded beta-sheet and four alpha-helices. The folding of the peptide fragment containing the zinc-binding motif HExxHxxGxxH bears only a distant resemblance to thermolysin, but is identical to that found in astacin, with the three histidines and a water molecule (linked to the glutamic acid) likewise constituting the zinc ligand; adamalysin II lacks a fifth (tyrosine) zinc ligand, however, leaving its zinc ion tetrahedrally co-ordinated. Furthermore, adamalysin II and astacin share an identical active-site basement formed by a common Metturn. Due to their virtually identical active-site environment and similar folding topology, the snake venom metalloproteinases (hitherto called adamalysins) and the astacins (and presumably also the matrix metalloproteinases/mammalian collagenases and the Serratia proteinase-like large bacterial proteinases) might be grouped into a common superfamily with distinct differences from the thermolysin family.

PMID: 8223430 [PubMed - indexed for MEDLINE]

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1: J Mol Biol. 1994 Jun 17;239(4):513-44.

Refined 2.0 A X-ray crystal structure of the snake venom zinc-endopeptidase adamalysin II. Primary and tertiary structure determination, refinement, molecular structure and comparison with astacin, collagenase and thermolysin.

Gomis-Ruth FX, Kress LF, Kellermann J, Mayr I, Lee X, Huber R, Bode W.

Max-Planck-Institut fur Biochemie, Martinsried, Germany.

Adamalysin II, alias proteinase II, a 24 kDa zinc-endopeptidase isolated from the snake venom of the Eastern diamondback rattlesnake Crotalus adamanteus, is a prototype of the proteolytic domain of snake venom metalloproteinases and of domains found in mammalian reproductive tract proteins. Its 2.0 A crystal and molecular structure was solved by multiple isomorphous replacement using six heavy-atom derivatives, and was refined to a crystallographic R-value of 0.172. 201 of the 203 amino acid residues of adamalysin II are defined by electron density; only the first two residues are disordered and crystallographically undefined in the crystal structure. Three-quarters of these crystallographic amino acid residue assignments were confirmed by chemical sequencing. In addition, the active-site zinc-ion, a hepta-coordinated calcium ion, a fixed sulphate anion and 173 solvent molecules were localized in the structure. Adamalysin II is an ellipsoidal molecule with a relatively flat active-site cleft separating the "upper" main body from a small "lower" subdomain. The regularly folded N-terminal upper domain consists essentially of a central, highly twisted five-stranded beta-pleated sheet flanked by a long and a short surface located helix on its convex side, and by two long helices, one of which represents the central "active site helix", on its concave side. The lower subdomain, comprising the last 50 residues, is organized in multiple turns, with the chain ending in a long C-terminal helix and an extended segment clamped to the upper domain via a disulphide bridge. The catalytic zinc-ion, located at the bottom of the active-site cleft, is almost tetrahedrally co-ordinated by His142, His146 and His152, and a water molecule anchored to an intermediate glutamic acid residue (Glu143), with the three imidazole N epsilon 2 nitrogen atoms 2.1 A and the solvent oxygen atom 2.4 A away from the zinc ion. His142, Glu143 and His146 are part of the long active-site helix, which extends up to Gly149, where it turns sharply away towards His152.

The importance of these residues for structure and activity of adamalysin II explains their occurrence in the HEXXHXXGXXH consensus sequence. Asp153, which is strictly conserved in these snake venom and reproductive tract metalloproteinases, is buried in the subdomain and seems to stabilize the hydrophobic active-site basement. Some residues behind, the adamalysin peptide chain folds into a characteristic 1,4-turn (the "Met-turn") containing the conserved Met166, which forms a hydrophobic basement for the three zinc-binding imidazoles.(ABSTRACT TRUNCATED AT 400 WORDS)

PMID: 8006965 [PubMed - indexed for MEDLINE]

1: Nature. 1997 Sep 4;389(6646):77-81. Related Articles, Links

nature

Mechanism of inhibition of the human matrix metalloproteinase stromelysin-1 by TIMP-1.

Gomis-Ruth FX, Maskos K, Betz M, Bergner A, Huber R, Suzuki K, Yoshida N, Nagase H, Brew K, Bourenkov GP, Bartunik H, Bode W.

Max-Planck-Institut fur Biochemie, Abteilung fur Strukturforschung, Martinsried, Germany.

Matrix metalloproteinases (MMPs) are zinc endopeptidases that are required for the degradation of extracellular matrix components during normal embryo development, morphogenesis and tissue remodelling. Their proteolytic activities are precisely regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs). Disruption of this balance results in diseases such as arthritis, atherosclerosis, tumour growth and metastasis. Here we report the crystal structure of an MMP-TIMP complex formed between the catalytic domain of human stromelysin-1 (MMP-3) and human TIMP-1. TIMP-1, a 184-residue protein, has the shape of an elongated, contiguous wedge. With its long edge, consisting of five different chain regions, it occupies the entire length of the active-site cleft of MMP-3. The central disulphide-linked segments Cys 1-Thr 2-Cys 3-Val 4 and Ser 68-Val 69 bind to either side of the catalytic zinc. Cys 1 bidentally coordinates this zinc, and the Thr-2 side chain extends into the large specificity pocket of MMP-3. This unusual architecture of the interface between MMP-3 and TIMP-1 suggests new possibilities for designing TIMP variants and synthetic MMP inhibitors with potential therapeutic applications.

PMID: 9288970 [PubMed - indexed for MEDLINE]

1: Eur J Biochem. 1995 Mar 15;228(3):830-41.

X-ray structures of human neutrophil collagenase complexed with peptide hydroxamate and peptide thiol inhibitors. Implications for substrate binding and rational drug design.

Grams F, Reinemer P, Powers JC, Kleine T, Pieper M, Tschesche H, Huber R, Bode W.

Max-Planck-Institut fur Biochemie, Martinsried, Germany.

Matrix metalloproteinases (MMPs) are a family of zinc endopeptidases involved in tissue remodeling. They have also been implicated in various disease processes including tumour invasion and joint destruction and are therefore attractive targets for inhibitor design. For rational drug design, information of inhibitor binding at the atomic level is essential. Recently, we have published the refined high-resolution crystal structure of the catalytic domain of human neutrophil collagenase (HNC) complexed with the inhibitor Pro-Leu-Gly-NHOH, which is a mimic for the unprimed (P3-P1) residues of a bound peptide substrate. We have now determined two additional HNC complexes formed with the thiol inhibitor HSCH2CH(CH2Ph)CO-L-Ala-Gly-NH2 and another hydroxamate inhibitor, HONHCOCH(iBu)CO-L-Ala-Gly-NH2, which were both refined to R-values of 0.183/0.198 at 0.240/0.225-nm resolution. The inhibitor thiol and hydroxamate groups ligand the catalytic zinc, giving rise to a slightly distorted tetrahedral and trigonal-bipyramidal coordination sphere, respectively. The thiol inhibitor diastereomer with S-configuration at the P1' residue (corresponding to an L-amino acid analog) binds to HNC. Its peptidyl moiety mimics binding of primed (P1'-P3') residues of the substrate. In combination with our first structure a continuous hexapeptide corresponding to a peptide substrate productively bound to HNC was constructed and energy-minimized. Proteolytic cleavage of this Michaelis complex is probably general base-catalyzed as proposed for thermolysin, i.e. a glutamate assists nucleophilic attack of a water molecule. Although there are many structural and mechanistic similarities to thermolysin, substrate binding to MMPs differs due to the interactions beyond S1'-P1'. While thermolysin binds substrates with a kink at P1', substrates are bound in an extended conformation in the collagenases. This property explains the tolerance of thermolysin for D-amino acid residues at the P1' position, in contrast to the collagenases. The third inhibitor,

HONHCOCH(iBu)CO-L-Ala-Gly-NH2, unexpectedly binds in a different manner than anticipated from its design and binding mode in thermolysin. Its hydroxamate group obviously interacts with the catalytic zinc in a favourable bidentate manner, but in contrast its isobutyl (iBu) side chain remains outside of the S1' pocket, presumably due to severe constraints imposed by the adjacent planar hydroxamate group. Instead, the C-terminal Ala-Gly-NH2 tail adopts a bent conformation and inserts into this S1' pocket, presumably in a non-optimized manner. Both the isobutyl side chain and the C-terminal peptide tail could be replaced by other, better fitting groups.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 7737183 [PubMed - indexed for MEDLINE]

1: Nature. 1989 Mar 16;338(6212):225-8.

nature

Structure of tumour necrosis factor.

Jones EY, Stuart DI, Walker NP.

Laboratory of Molecular Biophysics, Oxford, UK.

Tumour necrosis factor is a trimeric molecule, each subunit of which consists of an antiparallel beta-sandwich. Individual subunits from the trimer by a novel edge-to-face packing of beta-sheets. A comparison of the subunit fold with that of other proteins reveals a remarkable similarity to the 'jelly-roll' structural motif characteristic of viral coat proteins.

PMID: 2922050 [PubMed - indexed for MEDLINE]

1: Methods Enzymol. 1990;185:537-66. Related Articles, Links

Selection and coamplification of heterologous genes in mammalian cells.

Kaufman RJ.

PMID: 1974323 [PubMed - indexed for MEDLINE]

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1: FEBS Lett. 1997 Jan 6;400(3):333-5. Related Articles, Links

ELSEVIER SCIENCE FULL-TEXT ARTICLE

Purification of ADAM 10 from bovine spleen as a TNFalpha convertase.

Lunn CA, Fan X, Dalie B, Miller K, Zavodny PJ, Narula SK, Lundell D.

Department of Immunology, Schering-Plough Research Institute, Kenilworth, NJ 07033, USA. charles.lunn@spcorp.com

We have purified a protease with characteristics of TNFalpha convertase from bovine spleen membranes. Peptide sequencing of the purified protein identified it as ADAM 10 (Genbank accession no. Z21961). This metalloprotease cleaves a recombinant proTNFalpha substrate to mature TNFalpha, and can cleave a synthetic peptide substrate to yield the mature TNFalpha amino terminus in vitro. The enzyme is sensitive to a hydroxamate inhibitor of MMPs, but insensitive to phosphoramidon. In addition, cloned ADAM 10 mediates proTNFalpha processing in a processing-incompetent cell line.

PMID: 9009225 [PubMed - indexed for MEDLINE]